IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(s):	Florian Kern	CONF. NO.	5234
SERIAL NO.:	09/600,564	ART UNIT:	1645
FILING DATE:	November 7, 2000	EXAMINER:	Zeman, Robert A.
TITLE:	A Method for Identifying T-Cell Stimulating Protein Fragments		
ATTORNEY DOCKET NO.:	100725-9		

Commissioner for Patents
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DECLARATION OF FLORIAN KERN

1. My name is Florian Kern. I am a citizen of Germany. My residing at 168, Wick Hall, Furze Hill, Hove, BN3 1NJ, United Kingdom.

2. My educational background is in the field of Medicine. I obtained the degree(s) of Medical Doctor from University of Duesseldorf, Germany.

3. I am Professor of Immunology, head of a research unit at the Brighton and Sussex Medical School, University of Sussex, Brighton, United Kingdom. My research is involved in the design, including validation and standardization, and application of antigen-specific flow-cytometry assays. My vitae is shown in Exhibit I.

4. I am the principal author of more than 20 Medline indexed research articles in this field summarized in Exhibit II.

5. I have carefully studied the specification and was involved in the wording of the claims in the US patent application US 09/600564.

6. The method described in the claims has become a household method in many research labs following its first publication in Nature Medicine in 1998 [Kern-F et al., T-cell epitope mapping by flow-cytometry, Nature Medicine, 1998, Aug;4(8):975-8]

7. The method is based on the short term stimulation of T lymphocytes with peptides where the T lymphocytes are contained in a cell suspension. Peptides are added for stimulation. In order to be able to stimulate the T-cells, the peptides need to be uploaded onto class-I Major Histocompatibility Complex (MHC) molecules, because this is the only way they can be recognized by T lymphocytes via the T cell receptor (TCR). Loading of the peptides onto the MHC may require shortening (clipping) of peptides by some as yet not precisely identified proteolytic mechanism. The loading of peptides, including the clipping, onto class-I MHC molecules is known to be achieved within approximately 30 minutes.

8. It is known that once T-cells are being stimulated, they start synthesizing molecules which can be used to identify such stimulation. The production of these molecules, among which are cytokines, follows different kinetics. 6 hours is known to be a time after which most cytokines can be found, in particular IFN-gamma, IL-2, and TNF. No one single time-point is optimum for all cytokines; however, such cytokines will have reached reach a point of maximum secretion at approximately 12 hours following stimulation.

9. It is also known that, approximately 16 - 20 hours following

stimulation, T lymphocytes may start replicating their DNA content in preparation for a cell division.

10. Typically, cell division will not occur before 24 hours after stimulation.

11. The description of the method in the claims of USSN 09/600564 states that

> ...the time of incubation [cell suspension plus peptides] should be sufficiently long so that the protein fragment or fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules present on the cell surface, said taking up being sufficient when an unambiguous identification of stimulated T-cells is possible; and the incubation time of the suspensions containing T-cells with the protein fragment or fragments is sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T-cells do not occur...
>
> The specifications further teach that this incubation time can be 6 hours.

12. In light of my explanation of what is known to those skilled in the art, the description of the method in the specification of USSN 09/600564 gives sufficient guiding to anyone skilled in the art to perform the method claimed in USSN 09/600564. Specifically, setting up the assay with a 6 hour incubation time, and then working with longer and shorter incubation times will enable everybody to make use of the method and to find the optimum incubation period for their particular system.

13. The method claimed in US 09/600564 states that

> ...the time of incubation [cell suspension plus peptides] should be sufficiently long so that the

protein fragment or fragments are sufficiently taken up by the major histocompatibility antigen (MHC) molecules present on the cell surface, said taking up being sufficient when an unambiguous identification of stimulated T-cells is possible; and the incubation time of the suspensions containing T-cells with the protein fragment or fragments is sufficiently short so that selection and proliferation accompanied by the specific elimination of particular T-cells do not occur...

The specification further teaches that this incubation time can be 6 hours.

14. Based on the above explanation, I believe the description of the method gives sufficient guidance to anyone skilled in the art to perform the method. Specifically, setting up the assay with a 6 hour incubation time, and then working with longer and shorter incubation times will enable everybody to make use of the method and to find the optimum incubation period for their particular system.

15. Unlike the instantly claimed method, the publication by Yanagisawa et al. (Int. Immunology, 1997, Vol. 9, No.2, pp 227-237) describes a method as follows:

1. T-cells in a suspension are stimulated with antigens (proteins) from M. tuberculosis.

2. After 4 days of incubation proliferation of is examined by 3H thymidine incorporation of the cell suspension.

3. One of the proteins is identified as being the most stimulating.

4. Overlapping peptides are made to span said protein.

5. The incubation experiment is repeated with each single

peptide and proliferation measured after 3 days (72 hours).

6. One peptide is identified as having caused proliferation of the T-cells in the suspension.

7. Activation at the single cell level is not measured, not by flow-cytometry nor any other method.

8. Activated T-cells (cytokine or activation marker) are not identified.

9. T-cell proliferation is measured instead.

10. The read-out for T-cell proliferation is 3H thymidine incorporation.

11. Flow-cytometry is used exclusively to determine the composition of the cell suspension in regards of T-cell receptor Vbeta chain usage after the incubation period. Flow-cytometry is not used to determine T-cell activation markers. Determining the V-beta usage in the proliferated cells means that the cells are examined in regards of a component of the T-cell receptor that is derived from the V-region (V=Variability) of the T-cell receptor beta-chain genes. During T-cell development one of >50 of such V-segments is selected in each T-cell during somatic gene rearrangement, leading to the formation of a particular T-cell receptor. The determination of V-beta usage does not allow predicting the specificity of a T-cell or to measure activation.

16. The primary differences between the procedure of Yanagisawa et al. and the method described in US 09/600564 are as follows:

1. The incubation time with peptides in the instantly claimed method was selected to be short enough to *avoid* selection and proliferation, but proliferation is the mainstay of the method described by Yanagisawa et al.

2. Yanagisawa measures proliferation at a population level (3H thymidine incorporation). One or several short-term activation markers (e.g. cytokines) in the instantly claimed method are determined at the single cell level by flow-cytometry.

3. Peptides that induced rapid T-cell activation are identified by assigning stimulated T-cells to the peptides used for stimulation.

4. Yanagisawa use proliferation as their only experimental read-out to determine an effect of proteins or peptides on T-cells after 3-4 days of cell culture. In contrast to this, US 09/600564 totally avoids proliferation by using a short-term activation readout.

5. Yanagisawa uses flow-cytometry to characterize proliferated T-cells. The instant application uses flow-cytometry to detect T-cell activation.

17. Thus the scientific basis of the method of the instant application is clearly different from that of Yanagisawa.

18. There is no scientifically logical way how one would conclude from the publication by Yanagisawa that:

1. short term stimulation with soluble peptides will be

able to be detected by intracellular cytokine staining at the single cell level;

2. that such an approach will be sensitive; or

3. that such an approach is suitable to be used for epitope mapping.

19. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Declarant

12 Dec 2007
Date

Respectfully submitted,

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